Nova Hedwigia 50 3—4 355—360 Stuttgart, Mai 1990

Scleroderma citrinum (Gasteromycetes, Sclerodermatales) and Larix decidua form ectomycorrhizae in pure culture

by

Dana L. Richter and Johann N. Bruhn

School of Forestry and Wood Products
Michigan Technological University
Houghton, Michigan 49931

With 2 figures

Richter, D.L. & J.N. Bruhn (1990): *Scleroderma citrinum* (Gasteromycetes, Sclerodermatales) and *Larix decidua* form ectomycorrhizae in pure culture. - Nova Hedwigia 50: 355-360.

Abstract: Ectomycorrhizae were synthesized in pure culture between *Larix decidua* (European larch) and the gasteromycete, *Scleroderma citrinum*. Mycorrhizae were predominantly monopodal, having a pure white mantle and abundant attached mycelial strands. Mycorrhizae morphology is similar to that reported for other species of *Scleroderma* in combination with other hosts. This is a new symbiotic host-fungus combination reported in the literature, and extends the range of hosts with which *S. citrinum* is able to form mycorrhizae.

Introduction

Pure culture synthesis of mycorrhizae is an important technique for verification of symbiotic relationships between suspected mycorrhizal fungi and host trees (Trappe 1962, Harley & Smith 1983, Molina & Palmer 1982). Pure culture formation of mycorrhizae between known component species makes it possible to characterize morphological features of the established association. Knowledge of these features aids identification of native mycorrhizae, and can be used to determine effectiveness of seedling inoculation with specific mycorrhizal fungi (Trappe 1977). Determining that symbiosis between two organisms can exist, and describing important features of the structures they jointly produce, is basic to mycorrhizae research.

In the northern Great Lakes States of the U.S., species of the gasteromycete genus *Scleroderma* are found in association with both coniferous and deciduous trees on mesic to xeric sites (Richter & Bruhn 1987). As gasteromycetes, the basidiocarps of these fungi represent a source of abundant ectomycorrhizal spore inoculum. Several species of *Scleroderma* have been shown to form ectomycorrhizae in pure culture with *Pinus resinosa* Ait. (Richter & Bruhn 1986; Richter & Bruhn 1987). In addition,

0029-5035/90/0050-0355 $1.50
© 1990 J. Cramer in der Gebrüder Borntraeger
Verlagsbuchhandlung, D-1000 Berlin · D-7000 Stuttgart

we tested the ability of *Scleroderma citrinum* Pers. (= *S. aurantium* (Vaill.) Pers.) to form mycorrhizae in pure culture with another conifer of interest to the Great Lakes States forest industry, *Larix decidua* Mill. The purpose of this paper is to report the successful formation of ectomycorrhizae between this host-fungus combination.

Materials and methods

The isolate of *Scleroderma citrinum* was obtained from a fresh sporocarp collected in a 27 yr old red pine plantation. The site is a xeric sandy plain in Schoolcraft County, in the Upper Peninsula of Michigan, U.S.A. Voucher specimens of sporocarps were dried and have been retained in the Forest Microbiology Laboratory, Michigan Technological University, Houghton, Michigan, U.S.A.

Small pieces of interior sporocarp tissue were aseptically excised and cultured on Hagem's agar medium (Modess 1941) in a Petri dish. The isolate was maintained by transferring to fresh medium in test-tubes, allowing cultures to grow 30-45 da, refrigerating at 5 °C, and subculturing every 4-6 months until use in the mycorrhizae synthesis, 18 mo later. This same isolate was used in the successful synthesis of *Pinus resinosa* ectomycorrhizae (Richter & Bruhn 1986), and has been deposited in the American Type Culture Collection, Rockville, Maryland, U.S.A. (ATCC no 60969).

The pure culture synthesis method used was the same as that reported by Richter & Bruhn (1986), with only the following slight modifications. Rooting substrate per jar consisted of 300 ml vermiculite and ground sphagnum peat (10:1 v/v). One hundred fifty ml liquid MMN (Marx 1969) were added per vessel, followed by autoclaving for 30 min. Upon cooling, the pH of the substrate measured 4.5-5.0, but after several days, when seedlings were planted, the pH stabilized at 5.0-5.5.

Surface-sterilized *Larix decidua* seeds (Austrian stock) were plated on potato dextrose agar (PDA) in Petri dishes. The use of PDA allowed easier observation of occasional fungus contaminants than did water agar. Three uncontaminated seedlings were planted in each of 10 synthesis vessels. Seedlings were illuminated 18 hr/da under 4, 4 ft, 40 watt Westinghouse "Agro-lites"^{R} positioned approximately 20 cm above the vessels. The vessels were tipped at a 45° angle to directly illuminate their clear glass sides instead of the metal lids. Air temperatures surrounding the vessels ranged 15-20 °C dark period and 25-30 °C light period.

Inoculum and inoculation was carried out exactly as previously described (Richter & Bruhn 1986). Following inoculation vessels were sealed with Parafilm^{R} and returned to illumination. Four months following inoculation vessels were opened under sterile conditions and a portion of the substrate plated on MMN agar. Using sterile forceps, several mycorrhizae were separated from lateral roots, thoroughly rinsed in sterile water and plated on MMN agar. Finally, the entire seedling's root system was washed free of substrate and examined macro- and microscopically. Sizes of structures described are means of 5-10 measurements.

Results

In seven of the synthesis vessels, *Larix decidua* seedlings were green and healthy, attaining heights of 8-15 cm with root systems 12-20 cm long. In these vessels, mycorrhizae were formed on approximately 50% of the root tips, especially in the upper portions of the substrate closer to the inoculum. *Scleroderma citrinum* was reisolated from the substrate and from individual mycorrhizae in these vessels. The remaining three vessels had become contaminated with *Penicillium* spp. The seedlings in these vessels were chlorotic and moribund, with no mycorrhizae formed.

Individual mycorrhizae were distinctly white, glabrous to sparingly tomentose, with mantle mycelium often giving rise to concolorous mycelial strands (Fig. 1). Mycorrhizae were predominantly monopodal with rare bipodal forms, and measured 1-5 mm long by approximately 1 mm diameter. Root hairs were present on lateral roots and nonmycorrhizal root tips, but were absent from mycorrhizae (Fig. 1). Short sections of lateral roots were also slightly colonized with white *S. citrinum* mycelium (Fig. 1).

The mycorrhizal mantle, 20-50 μm thick, was composed of tightly bound hyphae. The mantle was thinnest to nearly lacking over the root apex, showing the pink epidermal rootcap cells underneath. Mantle hyphae were clear-walled, 3-6 μm diameter, with abundant clamp connections. Two clamps were commonly observed at branching points, one each on the main hypha and the branch hypha, just beyond the branch origin. Where inner mantle hyphae contacted root cortical cells, interdigitating hyphae formed a conspicuous "puzzle-like" pattern. Hartig net hyphae were 2-4 μm diameter, and penetrated between root cortical cells 3-4 cell layers inward.

Mycelial strands were composed of parallel hyphae with characteristic nodes along their length, especially where aggregations of clamps and branch strands occurred (Fig. 2). Mycelial strands averaged 0.1 to 0.25 mm diameter, but smaller aggregations of several intertwined parallel hyphae were common. Mycelial strands still attached to mycorrhizae after washing were up to 15 mm long, but these were undoubtedly fragmented during the washing process, and were likely much longer originally.

Discussion

Scleroderma citrinum (= *S. aurantium*) has been reported to be mycorrhizal with numerous coniferous and deciduous hosts. Trappe (1962) cites many possible host associations based on field observation reports, including *Larix decidua*. *Scleroderma citrinum* has been reported from coal mine spoils associated with *P. virginiana* Mill. in the U.S. (Schramm 1966) and *Betula* spp. in England (Ingleby et al. 1985). In Chile, Garrido (1984, 1986) reported *S. citrinum* to be common in nurseries and plantations of *P. radiata* D. Don, and also in plantations of *Eucalyptus globulus* Labill., *Populus nigra* L. and *Betula pendula* L. In the northern Great Lakes States of the U.S. we have observed *S. citrinum* associated with *Abies balsamea* (L.) Mill., *Betula allegheniensis* Britton, *B. papyrifera* Marsh, *Picea glauca* (Moench) Voss, *Pinus banksiana* Lamb., *P. resinosa, P. strobus* L., *Quercus rubra* L., and *Tsuga canadensis* (L.) Carr.

Individual reports of pure culture mycorrhizae synthesis with *S. citrinum* are reported for *Pinus patula* Schl. and Cham. (Kannan & Natarajan 1987), *P. radiata* (Malajczuk et al. 1982), and *P. resinosa* (Richter & Bruhn 1986). Mycorrhizae have been formed on greenhouse seedlings of *Quercus rubra* using spores of *S. citrinum* (Beckjord & McIntosh 1984). Trappe (1962) lists pure culture mycorrhizae syntheses

performed by various researchers with *Picea abies* (L.) Karst., *Pinus mugo* Turra., *P. strobus, P. sylvestris* L., and *P. virginiana,* but no reports with any *Larix* spp. Molina & Trappe (1982) report mycorrhizae synthesized between *S. hypogaeum* Zeller and *Larix occidentalis* Nutt. Since no literature reports have been encountered describing pure culture mycorrhizae formation by *S. citrinum* and *L. decidua,* we believe this to be a newly confirmed symbiotic association.

The morphologically distinctive white mycorrhizae with attached concolorous mycelial strands formed by *L. decidua* and *S. citrinum* have been described elsewhere for mycorrhizae formed by this fungus with other hosts, both coniferous (Richter & Bruhn 1986; Kannan & Natarajan 1987), and deciduous (Ingleby et al. 1985).

Several other species of *Scleroderma* also form similar mycorrhizae and mycelial strands with a variety of hosts. These were noted in syntheses between *P. resinosa* and *S. meridionale* Demo. and Malen. (= *S. macrorrhizon* Wall.), *S. cepa* Pers., and *S. polyrhizum* Pers., and also in a greenhouse inoculation of *P. resinosa* seedlings with *S. verrucosum* Vaill.: Pers. (Richter & Bruhn 1987). Morphologically similar mycorrhizae were also described for the combinations of *P. radiata* with *S. verrucosum* (Chu-Chou & Grace 1983), and *Carya illinoensis* (Wangenh.) K. Koch with *S. bovista* Fr. (Marx & Bryan 1969).

In addition to *Larix occidentalis* mentioned above, *S. hypogaeum* formed white mycorrhizae and mycelial strands with *Picea sitchensis* (Bong.) Carr., *Pseudotsuga menziesii* (Mirb.) Franco, *Tsuga heterophylla* (Raf.) Sarg., and three western U.S. *Pinus* spp. (Molina & Trappe 1982). Thus it seems ectomycorrhizae formed by *Scleroderma* spp. are characteristically white with attached concolorous mycelial strands regardless of the host involved.

Acknowledgements

The authors are grateful for financial support from the Michigan Department of Natural Resources, the Mead Paper Corporation, Escanaba, Michigan, and the Center for Intensive Forestry in Northern Regions, Michigan Technological University, Houghton, Michigan.

Literature

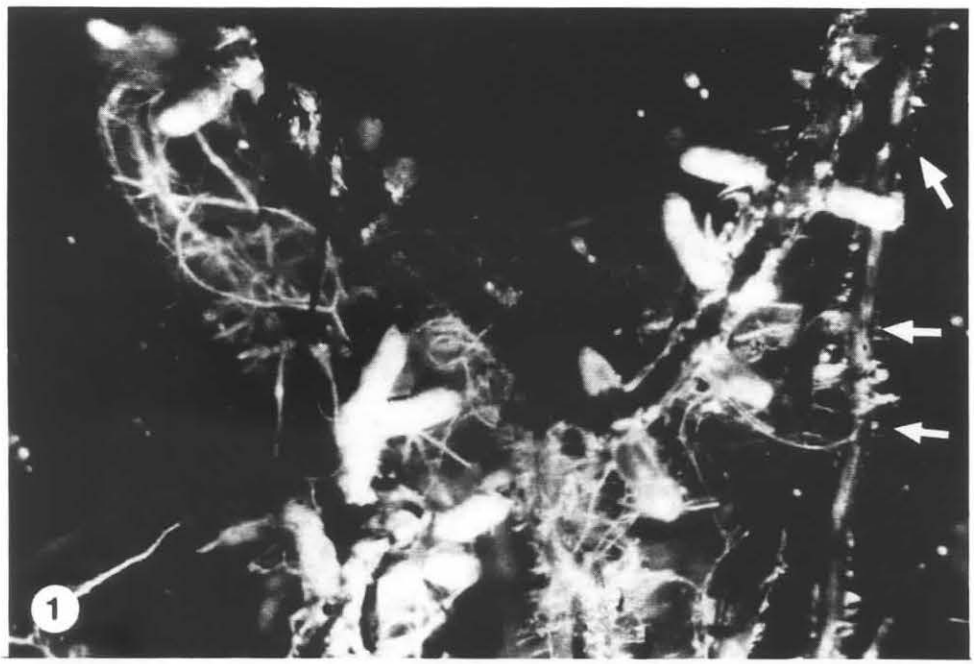
BECKJORD, P.R. & M.S. McINTOSH (1984): Growth and fungal persistence by *Quercus rubra* inoculated with ectomycorrhizal fungi and planted on a clear-cutting and strip mine. - Can. J. Bot. **62:** 1571-1574.

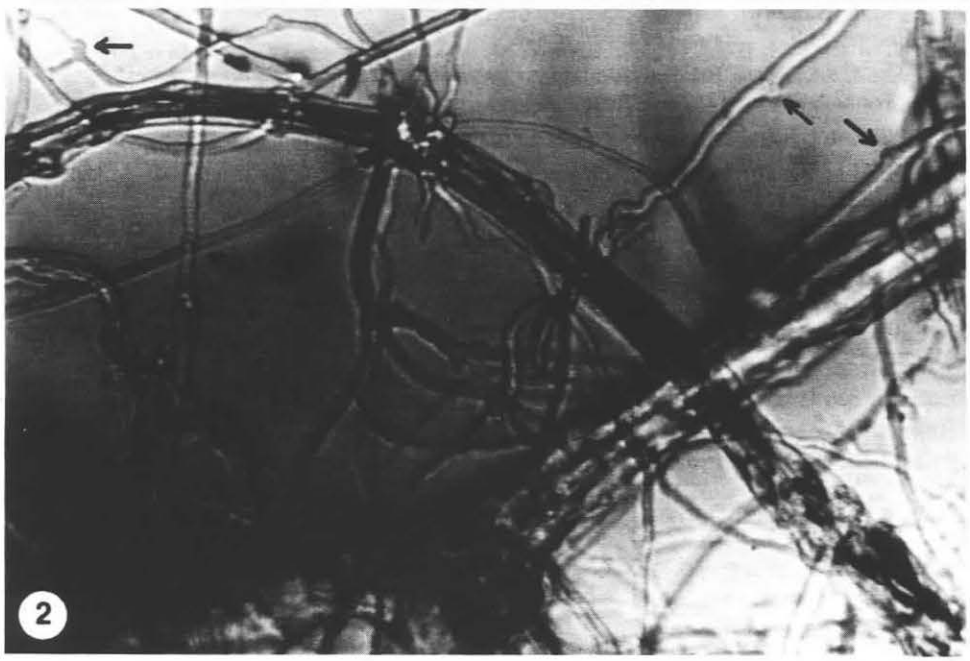
CHU-CHOU, M. & L.J. GRACE (1983): Characterization and identification of mycorrhizas of radiata pine in New Zealand. - Aust. For. Res. **13:** 121-132.

GARRIDO, N. (1984): Notes on *Scleroderma citrinum* Pers. (Mycota-Gasteromycetes) in *Pinus radiata* forests in Chile. - Nova Hedwigia **40:** 511-516.

GARRIDO, N. (1986): Survey of ectomycorrhizal fungi associated with exotic forest trees in Chile. - Nova Hedwigia **43:** 423-442.

HARLEY, J.L. & S.E. SMITH (1983): Mycorrhizal Symbiosis. - Academic Press, London.





Figs. 1-2: Ectomycorrhizae formed between *Scleroderma citrinum* and *Larix decidua*. Fig. 1. Low-power micrograph showing lateral roots bearing predominantly monopodal, white ectomycorrhizae with abundant mycelial strands. Note root hairs (arrows) on uncolonized lateral roots and portions of lateral roots lightly colonized with white mycelium. Approx. ×5. Fig. 2. Light micrograph of typical nodulose mycelial strands composed of parallel hyphae which bear numerous clamp connections (arrows). Approx ×333.

INGLEBY, K., F.T. LAST & P.A. MASON (1985): Vertical distribution and temperature relations of sheathing mycorrhizas of *Betula* spp. growing on coal spoil. - For. Ecol. and Mgmnt. **12:** 279-285.

KANNAN, K. & K. NATARAJAN (1987): Pure culture synthesis of *Pinus patula* ectomycorrhizae with *Scleroderma citrinum*. - Curr. Sci. **56:** 1066-1068.

MALAJCZUK, N., R. MOLINA & J.M. TRAPPE (1982): Ectomycorrhizae formation in *Eucalyptus*. I. Pure culture synthesis, host specificity and mycorrhizal compatibility with *Pinus radiata*. - New Phytol. **91:** 467-482.

MARX, D.H. (1969): The influence of ectotrophic mycorrhizal fungi on the resistance of pine roots to infection. I. Antagonism of mycorrhizal fungi to root pathogenic fungi and soil bacteria. - Phytopath. **59:** 153-163.

MARX, D.H. & W.C. BRYAN (1969): *Scleroderma bovista*, an ectotrophic mycorrhizal fungus of pecan. - Phytopath. **59:** 1128-1132.

MODESS, O. (1941): Zur Kenntnis der Mykorrhizabildner von Kiefer und Fichte. - Symb. Bot. Upsal. **5:** 1-146.

MOLINA, R. & J.G. PALMER (1982): Isolation, maintenance, and pure culture manipulation of ectomycorrhizal fungi. - In: SCHENCK, N.C. (ed.): Methods and Principles of Mycorrhizal Research: 115-129. - The American Phytopath. Soc., St. Paul, Minn.

MOLINA, R. & J.M. TRAPPE (1982): Patterns of ectomycorrhizal host specificity and potential among Pacific northwest conifers and fungi. - For. Sci. **28:** 423-458.

RICHTER, D.L. & J.N. BRUHN (1986): Pure culture synthesis of *Pinus resinosa* ectomycorrhizae with *Scleroderma aurantium*. - Mycologia **78:** 139-142.

RICHTER, D.L. & J.N. BRUHN (1987): *Scleroderma* spp. ectomycorrhizae for use in greenhouse and nursery to increase *Pinus resinosa* outplanting success. - Mycol. Soc. Am. Newsletter **38:** 45 (Abstract, Ann. Meeting. M.S.A. Ottawa, Ontario, Canada. June 22-25, 1987).

SCHRAMM, J.R. (1966): Plant colonization studies on black wastes from anthracite mining in Pennsylvania. - Trans. Amer. Phil. Soc. 56 (Pt. 1): 1-194.

TRAPPE, J.M. (1962): Fungus associates of ectotrophic mycorrhizae. - Bot. Rev. **28:** 538-606.

TRAPPE, J.M. (1977): Selection of fungi for ectomycorrhizal inoculation in nurseries. - Ann. Rev. Phytopath. **15:** 203-222.